Assessment of mortality sources on diamondback terrapins (Malaclemys terrapin) in the Charleston Harbor estuary

Annual Report To U.S. Fish and Wildlife Service









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ANNUAL REPORT TO THE U.S. FISH AND WILDLIFE SERVICE

For

Assessment of mortality sources on diamondback terrapins (*Malaclemys terrapin*) in the Charleston Harbor estuary.

by

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EXECUTIVE SUMMARY

In support of this research, nearly 100 days were logged in the field or laboratory in 2014 by SCDNR personnel, a graduate student at the College of Charleston, and cadre of volunteers.

Approximately 20% of annual effort was associated with receiving and processing 157 terrapins collected at 23 trammel net stations in the Ashley River (and two sites in Orange Grove Creek) between March and November. Processing of these terrapins included collecting morphometric data, a macroscopic physical examination, permanent scute marking, external disc tagging, opportunistic examination of fecal matter, and ultrasound examination of females as appropriate.

In addition to conventional marking and tagging, a subset of seven females and five males was tagged with acoustic transmitters to investigate spatio-temporal distribution patterns, with continued emphasis on movement between creek and river habitats and among river habitats. Nineteen terrapins captured in 2014 were previously tagged/marked; nine were tagged in 2009 by a graduate student at the College of Charleston, another nine were tagged in year one of this study, and one was recaptured twice in 2014 (once in our study, the second time by a member of the general public 10 km away). In addition, one dead terrapin was re-sighted and also reported by a member of the general public; visible external tags contributed to both public reportings.

Approximately 20% of annual effort was associated with maintaining and uploading data from 21 acoustic receivers deployed in the Ashley River and adjacent creeks. Between 1 January and 18 December 2014, a total of 21,614 detections were recorded by these receivers for twelve terrapins tagged in 2014 and seven (of 21) terrapins tagged in 2013 that continued to transmit. Ninety-five percent of detections occurred at receivers located within 0.5 km of where terrapins were originally captured; however, movement up to 10 km from capture sites also occurred.

Roughly 20% of annual effort was associated with infrastructure support and recording direct observation of terrapin behavior during interactions with crab trap funnels with and without three different by-catch excluder device (BRD) designs at the South Carolina Aquarium (SCA). Preliminary data confirm the efficacy of a design conceived to exclude 100% of terrapins based on body dimensions; however, behavioral response by crabs to this BRD design is still pending. Visual observations also confirm that roughly half of male terrapins evaluated will rotate their body to enter a crab trap; thus, the vertical orientation BRD is not a perfect terrapin solution.

And lastly, nearly 40% of annual effort in 2014 was associated with field testing the same BRD designs evaluated for terrapins at the SCA. During 362 crab trap sets (1,617 soak hours), a total of 1,341 blue crabs and 11 terrapins were captured across three primary fishing areas in the Ashley River near Duck Island, Old Towne and Orange Grove Creeks, and the Stono River. Statistical differences in catch rate for legal-sized blue crabs (≥5" or 12.7 cm) and overall size distribution was only detected between crab traps fished without BRDs and crab traps fished with the reduced height BRD design that was 100% effective at terrapin exclusion at the SCA.

Taken collectively, data collected for terrapins at the SCA in Job 2 and for terrapins and blue crabs collected in the field in Job 3, we are cautiously optimistic about moving forward with more rigorous testing of the vertical orientation BRD design with commercial crabbers in 2015.

Introduction

Diamondback terrapins (*Malaclemys terrapin*) are the only exclusively estuarine turtle in North America (Wood, 1977) and are distributed between Massachusetts and Texas. Maximum carapace length is <30 cm (Ernst et al., 1994) and morphological differences between larger females and smaller males (Ernst et al., 1994) minimize foraging niche overlap between the sexes (Tucker et al., 1995; Levesque, 2000). In the late 1800's, diamondback terrapins were prized table fare; however, that commercial market crashed within a few decades following overharvesting (Carr, 1952). This long-lived species (Hildebrand, 1932) remains globally listed at low but near threatened risk (IUCN; http://www.iucnredlist.org/details/12695/0). In South Carolina, diamondback terrapins are one of 52 species in the reptile/amphibian guild that is listed as a species of concern (SC CWCS 2005, p. 2-11).

No commercial terrapin fishery exists in South Carolina, but recreational "possession" of two terrapins per person (SC Code, Chapter 5, Article 23, Section 50-5-2300A). Despite no legal harvest, fisheries mortality continues to occur annually in South Carolina as a result of incidental capture and subsequent drowning in crab traps (Roosenburg et al., 1997; Dorcas et al., 2007; Grosse et al., 2011). Incidental capture rates for diamondback terrapins in South Carolina waters are unknown, but up to 78% of a local population has been estimated to be captured annually in other regions (Roosenburg et al., 1997). Despite this observation, mitigation measures to minimize diamondback terrapin entry into crab traps have not been mandated in South Carolina (in contrast to some mid-Atlantic states) due to associated reductions in absolute crab catches (despite statistically similar catch rates) based on SC field studies to date (Powers et al. 2009a).

The development and implementation of "long-term coastwide standardized surveys to estimate the abundance and distribution of South Carolina's terrapin population" is a top Conservation Action for this species of concern in the South Carolina Wildlife Action Plan (SC SWAP, 2015). Diamondback terrapins are the fifth most frequently encountered species captured in a coastwide trammel net survey designed to monitor inshore fisheries (Arnott et al., 2013), and more than 18,000 diamondback terrapin collections have occurred in this survey since 1995¹. Pronounced seasonal variability in catch rates is noted, with peak captures in the spring (Arnott et al., 2013) concurrent with peak incidental catch rates in commercial crab traps (Powers et al., 2009a). Spatial and temporal disparity in catch rates was also reported. For example, catch rates in the Ashley River were twice as great as most other survey areas and relatively stable across years, whereas catch rates in the Wando River (which has been heavily developed but also historically had lower catch rates than the Ashley River; Bishop, 1983) steadily declined after 1995, consistent with the statewide trend (Arnott et al., 2013).

Telemetry data collected in the Ashley River in 2013 (year one of this study) indicate that diamondback terrapins disproportionately occur in river vs. tidal creek habitats near Duck Island. Following aggregation and frequent acoustic detection in spring, diamondback terrapins became more dispersed, with some individuals emigrating up to 10 km from the Duck Island study area. Frequent occurrence and routine movements within river habitats differed greatly from reports of high site fidelity to creek habitats (Spivey, 1998; Gibbons et al., 2001; Estep, 2005), which may also reflect differential vulnerability to in-water mortality sources than reported for more resident

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¹ Unpublished data. Inshore Fisheries Section, Marine Resources Research Institute, South Carolina Department of Natural Resources. Data provided by Dr. Steve Arnott, Principal Investigator.

terrapin populations. Therefore, the purpose of the second year of this study was three-fold. First, continued and expanded efforts were needed to better characterize the spatio-temporal distribution patterns of both male and female terrapins throughout the Ashley River (Job 1). Specifically, for Job 1 we set out to test the following two null hypotheses.

Ho: There is no difference in the proportion of time detected at Duck Island receivers as compared to receivers located at other locations in the Ashley River.

Ho: There is no difference in the recapture rate of diamondback terrapins captured at sites near Duck Island relative to other capture locations in the Ashley River.

Second, because the greatest in-water threat to diamondback terrapin populations appears to be drowning in fishing gears such as crab and eel pots and/or vehicular trauma in terrestrial realms (Dorcas et al., 2007; Grosse et al., 2011), research efforts in year two included emphasis on designing a more appropriate terrapin by-catch reduction device (BRD). Re-configuration of traditional BRD designs was made after considering body dimension of both blue crabs (*Callinectes sapidus*) and diamondback terrapins and their perceived funnel entry behavior. Laboratory (Job 2) and field data collection (Job 3) were used to evaluate the effectiveness of BRD design modifications as defined by the following null hypotheses:

Ho: There is no difference in the retention rate of diamondback terrapins in crab traps without, turtle excluder devices (TEDs) and crab traps with modified TEDs.

Ho: There is no difference in the retention rate of blue crabs in crab traps without, turtle excluder devices (TEDs) and crab traps with modified TEDs.

Lastly, we also proposed to use wildlife cameras to collect data to test two additional hypotheses about terrapin and predator occurrence at potential nesting sites on Duck Island, a location where depredated nests were documented in 2009. However, an expedition to this island in February 2014 did not reveal any suitable nesting habitat, suggesting that reproductively-active terrapins captured near this location (as observed in 2013 and again in 2014) likely nest elsewhere. As such, this aspect of our proposed research was not pursued further in 2014. Nevertheless, efforts to document (through public input and field expeditions) diamondback terrapin nesting events along the Ashley River and elsewhere remains a high research priority for future funding cycles.

Methods

Job 1. Spatio-temporal distribution patterns

Study site description

Job 1 was conducted in the Ashley River, the southernmost tributary of Charleston Harbor. Within the Ashley River watershed, the South Carolina Department of Health and Environmental Control (SCDHEC) reports 3,017.2 acres of estuarine habitat, and surface water quality is most often listed as "SA" (https://www.scdhec.gov/environment/water/shed/docs/50202-040.pdf). We selected the Ashley River for this study given that it has consistently supported the highest diamondback terrapin catch rates of all estuaries sampled since 1995 (Arnott et al., 2013), which

increased the probability of capturing adequate sample sizes for this study. Relative stability in annualized catch rates in the Ashley River since 1995 (Arnott et al., 2013) also suggested that the data collected during this telemetry study were more likely to reflect representative behaviors rather than a recent response to changing population structure.

Within the Ashley River, we initially (2013) selected five trammel net stations located near Duck Island, a large hummock island located on the south/west side of the river and situated nearly equidistant between the freshwater/saltwater dividing line and the Atlantic Ocean (Figure 1). Watershed development on the south/west side of the Ashley River near Duck Island is predominantly of a residential nature, whereas development on the opposite side of the river (where the SCDHEC maintains a water quality monitoring station) is largely industrial.

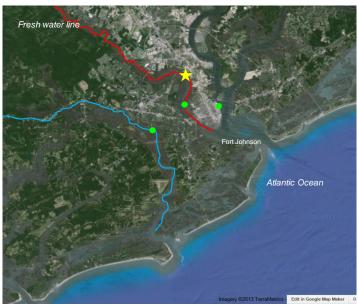


Figure 1. Research activities initiated in 2013 in the Ashley River (red line) near Duck Island (yellow star) were continued in 2014; however, additional activities (green dots) were added elsewhere in the Ashley River, the Stono River (blue line), and the South Carolina Aquarium.

Capture and general processing

Diamondback terrapins collected for this study were primarily captured by trammel netting at locations near Duck Island that were randomly selected each month. However, in 2014, capture locations were periodically expanded to include all sites in the Ashley River (Appendix 1) to increase the overall sample size and spatial distribution of marked individuals. Spatial expansion of capture locations also slightly increased the probability of recapturing any of the >1,200 terrapins tagged or marked in conjunction with six graduate studies (Levesque, 2000; Lee, 2003; Hauswaldt, 2004; Estep, 2005; Schwenter, 2007; Broyles, 2010) between 2000 and 2009.

The trammel net (Figure 2a) is a 600 ft. monofilament net consisting of multiple panels with a minimum mesh size of 4" (stretch), which is rapidly deployed from the stern of a skiff along ~450' of shoreline as described by Arnott et al. (2013). After an initial soak of approximately five minutes, during which time noise is used to 'spook' fish and terrapins from the marsh grass into the net, it is steadily retrieved over a period of about 20 minutes.





Figure 2. Diamondback terrapins were predominantly captured using a 600' trammel net (A), but some terrapins were also captured with wire mesh crab traps (B) in Job 3 in 2014.

Captured terrapins were externally marked with a grease pencil to differentiate among capture locations while they were held in aggregate in a large, ventilated plastic bin which was partially covered to provide shade. With few exceptions, data were collected after terrapin transport to a climate-controlled, shore-based facility; terrapins were held overnight and released as close as possible to their capture locations the next day. Standard data collection included:

- Visually inspect terrapins and note pre-existing marks and injuries
- Assess sex based on dimorphism (head/body size, cloaca position/tail length)
- Scan all soft tissue for the presence of a PIT tag
- Measure (cm) carapace length, width, and body depth with calipers (Haglof, Sweeden)
- Record body mass (kg) using a digital spring-scale
- Photograph dorsal, ventral, and lateral perspectives
- Drill small holes in marginal scutes using a multi-letter combination coding system
- Attach flexible shellfish tags (Floy Tag, Inc.; Seattle, WA) to the carapace using epoxy
- Assess reproductive condition of females using ultrasound
- Opportunistically collect and exam fecal matter to identify forage items

Acoustic transmitter attachment

Because sexual dimorphism reduces foraging niche overlap (Tucker et al., 1995) and because prior to this study very little multi-season habitat use data were available for male diamondback terrapins (Harden and Southwood Williard, 2012; Tulipani, 2013), we wished to study males as well as females. In 2014, the sampling design goal was to attach acoustic transmitters to 12 adult (mixed sex) diamondback terrapins captured near Duck Island and 10 adult (mixed sex) terrapins captured within Orange Grove Creek. In addition, we also hoped to attach three short signal repeat duration (30-sec vs. 4-min) transmitters to terrapins in order to manually track them and thus document the extent of tidally-mediated excursions between river and creek habitats.

Prior to attachment, barnacles and encrusting bryozoans were gently scraped from the vertebral and costal scutes while fine-scale organic matter such as algae was scrubbed off using alcohol-soaked gauze pads. Loose keratin scutes were carefully peeled away and 100-grit sandpaper was lightly applied to ensure no loose keratin remained.

Acoustic transmitters (V9-2H; Amirix Systems, Inc.) were attached to the second vertebral scute, but offset from center due to the vertical relief associated with vertebral scutes. A base layer of epoxy was first pressed to the carapace across at least three scute seams to minimize the risk of

transmitter loss due to detachment of a single scute. The epoxy was molded into a shape that had a centralized dome. Next, the transmitter was pressed onto the epoxy dome with the transducer end of the transmitter facing towards the rear of the animal. In "pig in a blanket" fashion, epoxy was then applied over top of the transmitter and blended evenly (Figure 3).



Figure 3. Acoustic transmitters were attached to the carapace of diamondback terrapins using a two-part putty epoxy spread across multiple scutes to increase retention duration.

Acoustic transmitters measured 9 mm (diameter) by 29 mm (length) and weighed 4.7 g in air (2.9 g in water). Approximately 9.5 g of a two-part epoxy putty (SonicWeld™; Ed Greene and Company) was used to secure these transmitters to the carapace. This use of a quick-setting epoxy to attach transmitters is standard for most diamondback terrapin telemetry studies conducted to date (Spivey, 1998; Butler, 2002; Harden and Southwood Williard, 2012), and has resulted in transmitter retention for nearly a year. For male diamondback terrapins, the total weight associated with the transmitter plus epoxy exceeded the 2% of body weight rule suggested by Winter (1996), but was comparable to transmitter package weights (4 to 39 g) previously reports for this species (Spivey, 1998; Harden and Southwood Williard, 2012). To minimize excessive drag, transmitters were only attached to males ≥300 g (Tulipani, 2013).

Acoustic telemetry data collection

Acoustic transmitters emitted coded signals (which distinguished individual terrapins) on a frequency of 69.0 kHz at random intervals between 180 and 300 seconds.

VR2W receivers (Amirix Systems, Inc.) were deployed near the water surface in a PVC (Sch 40) housing that floated, with the hydrophone end of the receiver facing downward (Figure 4a). This housing slid up and down a 3.8 cm diameter galvanized pole that measured 4.3 to 6.4 m in length depending on water depth and sediment consistency. Housing buoyancy was provided by an air and foam-filled PVC base and later augmented by surface-oriented air-filled PVC tubes. A 5.1 cm diameter PVC pole that measured 4.6 to 6.1 m in length (<1.5 m exposed at high tide) was positioned behind each galvanized pole to restrict the receiver housing from swaying during water level changes, as well as to mark the site during the highest water levels.

Acoustically-tagged terrapins were also detectable from the research vessel using a VR60 receiver and omni (VH65) and directional (V10) hydrophones (Figure 4b). This boat-based system enabled searches for acoustically-tagged terrapins to be conducted in areas outside of the reception range (estimated to be <200 m) from the fixed site VR2Ws, such as in the upper reaches and bends of creeks where direct line-of-sight fetch was limited.





Figure 4. Acoustic detections were recorded by VR2W receivers deployed in buoyant PVC housings (A), as well as opportunistically by a VR60 receiver and hydrophone system (B) during visits to the study area.

In 2014, VR2W acoustic receivers remained deployed at 13 locations first monitored in 2013, but were expanded on 14 March to include five locations associated with Orange Grove Creek (OGC, Figure 5) and again on 02 May to include two locations near the RT-7 (Cosgrove) bridge across the river from Duck Island as well as the main stem of the Ashley River between the Duck Island and OGC receivers. Three OGC receivers were lost (and never recovered despite efforts) prior to 04 April as a result of defective stainless steel hose clamps and the surviving receivers were pulled as a precaution; monitoring at all five OGC sites resumed on 30 May.



Figure 5. Twenty-one VR2W receivers were used to monitor acoustically-tagged diamondback terrapins in the Ashley River in 2014; 13 locations were established in 2013 (yellow dots) predominantly near Duck Island, with eight new locations (red dots) predominantly associated with Orange Grove Creek established in 2014.

Acoustic receivers provided continuous monitoring capability when submerged; however, because of the tidal nature of this study area, all receivers were exposed at low tide (Figure 6). Nonetheless, trammel nets can only be deployed when sufficient water levels are present; thus, the VR2W receivers monitored the entire tidal window of opportunity that trammel net sampling could occur each day of the study. VR2W receivers were removed from PVC housings every four to six weeks and the data were uploaded to a laptop computer using the VUE software and a Bluetooth USB connection. Concurrent with data uploading, biogenic fouling was removed from the PVC housing. Prior to redeployment ~10 minutes later, lithium grease was re-applied to the threaded fittings for the PVC housing cap and the stopper coupling on the galvanized pole, to assist with future data uploading missions.



Figure 6. VR2W receivers provided continuous monitoring coverage when submerged (A), but were exposed at low tide (B) due to large tidal amplitudes typical of estuaries in this region.

CTD data

A Levelogger Junior (Solinst, Inc.) CTD was deployed at the central VR2W receiver location (SCB) in the South Creek to record water temperature (°C) and conductivity (uS cm⁻¹) data at 15-minute intervals. Conductivity was converted to salinity (ppt) with the "Stevens EC to Salinity" spreadsheet provided by D. Sanger (SCDNR/MRD/MRRI; sangerd@dnr.sc.gov) that includes the following methods description: "Salinity is calculated from the un-normalised or normalised conductivity according to the algorithm outlined in Standard Methods for the Examination of Water and Wastewater, 18th Edition, p. 2-47. The equation for Rt was taken from "Specific Conductance: Theoretical considerations and application to analytical quality control" by R.L. Miller, W.L. Bradford, and N.E. Peters. United States Geological Survey Water-Supply Paper 2311. 1988. USGS, Federal Center Box 25425, Denver, CO 80225. A value of 1.84 %/ deg C is used as the un-normalising factor (the same value used in the EC200 to normalise). "

Statistical analyses

For all study Jobs reported herein, statistical tests assumed a significance level of $\alpha=0.05$ and were performed in Minitab $15^{\$}$ (Minitab, Inc.). In Job 1, correlation was used to test for statistical differences in water temperature and salinity across years, and, as appropriate, between these parameters and monthly acoustic detection of terrapins. In Job 2, correlation was used to test for differences in water temperature and salinity and interest in crab traps. In Job 3, neither blue crab catch rate nor size distribution data were normally distributed; thus, non-parametric Kruskal-Wallis rank comparison tests were used to evaluate catch rates and size distributions between crab traps fished without BRDs and crab traps fished with different BRD designs.

Job 2. Laboratory evaluation of BRD efficacy

This aspect of data collection was delegated to a graduate student (Janelle Johnson) at the College of Charleston who developed six general thesis questions as follows:

- (a) Are terrapins less capable of entering a crab trap with any of three BRD designs?
- (b) Are crabs less capable of entering a crab trap with any of three BRD designs?
- (c) Are crabs less capable of escaping a crab trap with any of three BRD designs?
- (d) Does the presence of terrapins in a crab trap encourage further terrapin entry?
- (e) Does the presence of terrapins in a crab trap discourage further crab entry?
- (f) Can an escape panel be fashioned to permit terrapin escapement but crab retention?

During this reporting period, data were only collected to answer the first research question; thus, the bulk of analyses for this Job will be included in the year three report for this research grant.

Capture, handling, and husbandry

All procedures described herein were approved by the College Of Charleston's Institutional Animal Care and Use Committee (IACUC), permit #2014-002.

All terrapins used in this study were captured by trammel netting in the Ashley River and processed as described in Job 1, with the exception that scute marking was conducted after terrapins had completed trials to ensure that this procedure did not alter behavior. Given their social nature and space limitations, terrapins were held in a 3 m diameter fiberglass holding tank where the majority of their time in captivity was spent. The holding tank contained structured habitat (i.e., cinder blocks, plastic tubes, and a floating material) to facilitate refuge and basking.

While in captivity, terrapins were fed a varied diet of teleosts (Mackerel) and invertebrates (shrimp, squid, clam) in the amount of 1-3% of body weight on non-observation days, but fasted for ~17 hours prior to observation trials to increase interest in trial bait. Terrapin weight was monitored several times monthly to ensure that optimum weight was maintained.

The holding tank was hydro-cleaned as necessary to remove debris from the bottom of the tank. Water quality (NH₃, NO₂, pH, alkalinity and salinity) was tested weekly, with more frequent monitoring if ammonia levels exceeded 1 ppm and/or nitrites exceeded 0.5 ppm. Target salinity was 12 to 17 ppt, and a chiller was activated as needed to keep water temperature below 32°C. Water changes occurred as needed, and sediment filters cleaned at filter pressures \geq 20 PSI.

Data collection

To evaluate BRD efficacy, terrapins were randomly removed from the holding tank and placed in an experimental tank (Figure 7) that consisted of a single crab trap side panel that featured a funnel with no BRD (control) or one of three different BRD designs (treatments). Terrapins were randomly assigned a number, then trials were conducted in chronological terrapin order while alternating between male and female terrapins. The panel was secured near one end of the 1.25 x 0.5 m trial tank via cinder blocks, with additional paneling used to prevent terrapin passage around the side or over the top of the primary crab trap panel. In addition to spatial constriction, water level was minimized to encourage terrapin investigation of the trap funnel in order to reach the Mackerel bait just on the other side of the panel.

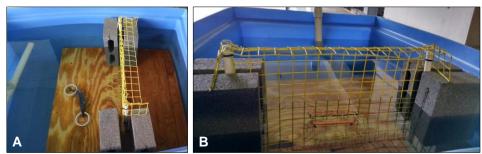


Figure 7. Direct observation of BRD efficacy was conducted in an experimental tank that encouraged terrapin investigation of a crab trap funnel in order to reach bait on the other side. Top-down/lateral (A) and longitudinal (B) views of this experimental tank are shown here.

The control panel (Figure 8a) was distinguished by a funnel sans BRD. The first treatment was a standard BRD (5.1 cm x 15.2 cm; Figure 8b) produced by Top-ME (Topsham, ME); this and all BRDs were inserted into the entry funnel and secured with plastic cable ties. The remaining two treatments were modifications of treatment one. Treatment two (Figure 8c), herein referred to as the "reduced height" BRD, was made by drilling two holes on each side of the BRD that were 1.0 cm (3/8 in.) below the 'ceiling' of the BRD, then inserting a 16.5 cm (6 $\frac{1}{2}$ ") long section of brass bar (diameter = 0.5 cm; $\frac{3}{16}$ "); this brass bar was then secured using epoxy (Powers T-308 and/or Sonic Weld). The final treatment (Figure 8d) was created by cutting the standard BRD along the longitudinal axis, connecting it back together with plastic cable ties, then rotating it 90°, herein referred to as the "vertical orientation" BRD.

Observation trials began the instant that each terrapin was placed in the non-baited side of the experimental tank, and were terminated when one of three conditions was met: (a) the terrapin successfully reached the bait; (b) 30 attempts to pass through the funnel occurred to no avail; (c) 30 minutes elapsed even if the terrapin made no effort to reach the bait. Water temperature and conductivity (converted to salinity, Job 1) in this tank were logged every 15-min with a CTD. Any contact with the trap panel as well as the funnel was recorded to gauge activity during trials.

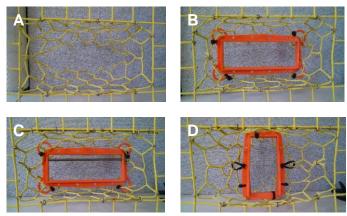


Figure 8. Efficacy of by-catch reduction devices (BRDs) for excluding terrapins from crab traps was evaluated by quantifying effort required to achieve successful passage through trap funnels without BRDs (A), with a commercially available BRD (B), and two modifications to this BRD that excluded terrapins by reducing the vertical opening size (C) or requiring them to rotate their body 90° (D) in order to enter the trap.

Job 3. Field evaluation of BRD efficacy

Crab trap fishing locations and deployment methodology

Crab trap fishing was conducted at 24 locations in the greater Charleston, SC area (Figure 9) in water body sizes ranging from river to small tidal creek. Given tidal amplitudes associated with these creeks and a desire to not leave traps unattended, traps were only fished in small tidal creeks during periods with sufficient water volume for boat access at low tide.

Traps were baited with a single Menhaden and fished in clusters of four that included a control trap and each of three treatments described in Job 2. Four colors (black, red, yellow, light green) of traps were fished; initially all four colors were fished as a cluster, but this practice was later changed to ensure that each cluster of four traps included four different colors to eliminate auto-correlation between color and fishing location. Each trap constituted a sampling event, with each trap retrieval time denoted with a sub-level code; this practice allowed for later examination of within-event variation of catch rates as so desired. Traps were generally fished as close to the edge of the marsh as possible, based on anecdotal reports from several crabbers that this practice is associated with increased terrapin catch. Target soak time was one hour before pulling traps for examination; this threshold was considered a compromise between trap disturbances that could discourage crab entry while simultaneously decreasing the risk of terrapin drowning.



Figure 9. Crab traps were fished in clusters of four (control plus three treatments) at 24 different locations in three areas: Ashley River near Duck Island (yellow fill); Orange Grove and Old Towne Creeks (orange fill); and the Stono River (green fill) within 2 km of Elliott's Cut.

Data collection

When captured, the number of crabs and terrapins in each trap was recorded as well as their relative position within the trap (i.e., in the lower or upper chamber). Each crab and terrapin was assigned a sequential project identification number. Terrapin data collection was the same as described in Job 1. Three caliper measurements (to nearest 0.1 cm) were recorded for crabs: carapace width (CW); carapace length (CL); and body depth (BD). Crabs were marked with nail polish (color and body location varied by sampling date) to document potential recapture events.

Results

Job 1. Distribution patterns of diamondback terrapins in the Ashley River

Capture and recapture

One hundred sixty-five (of 244) diamondback terrapins captured by monthly trammel net sets in the Ashley River between March and November were examined, measured, and marked/tagged. Forty-six percent (76) of terrapins processed in 2014 were captured between the I-526 and RT-7 bridges (Figure 5). Thirty-two percent (49) of terrapins were captured at five acoustically-monitored trammel net sites near Duck Island, with the remainder (30; 19%) captured downriver from the Charleston Rifle Club (Figure 5). Two additional terrapins in this 'lower zone' were captured by trammel netting (four sets) in tributaries of Orange Grove Creek on 10 April.

The total number of tagged and recaptured terrapins in 2014 was insufficient to formally test the null hypothesis of no difference in the recapture rate of diamondback terrapins captured at sites near Duck Island relative to other capture locations in the Ashley River. Nine of 128 (6.3%) diamondback terrapins marked/tagged in 2013 were recaptured in 2014, with a mean time at large of 389.4 days (range = 268 to 495 days). Only one terrapin captured in the trammel net survey in 2014 was recaptured; MT0221 was captured on 26 August at AR11, recaptured 23 October at AR09, and recaptured again in a cast net at the James Island Yacht Club (10 km away) on 27 October. A second public reporting of a tagged terrapin occurred when MT0136 was found dead on 24 May (45 days post-capture) at the Charleston Rifle Club (0.5 km from capture site). The cause of death for this terrapin is not known, but decapitation along a shoreline suggests a raccoon.

Nine diamondback terrapins previously tagged by Broyles (2010) were recaptured in 2014, four of which were recaptured near Duck Island. Since the present study began in 2013, 15 terrapins previously tagged by Broyles (2010) have been recaptured.

Demographics and health

Sixty-one percent of trammel net-processed diamondback terrapins were males (95) that measured 11.6 ± 0.1 (mean \pm standard error, SE) whereas female terrapins (61 measurements) were 15.5 ± 0.3 (mean \pm SE). Sixteen of 24 female terrapins captured between March and May received an ultrasound examination, with definitive follicles noted for only three individuals. However, among 10 females captured in April and transported to the South Carolina Aquarium (SCA) for BRD testing in Job 2, nine eventually deposited eggs (81) in captivity that were later reared under controlled conditions in a companion SWG study (Levesque & Grosse - PIs). Qualitative examination of 33 fecal samples (26 female, 7 male) revealed similar results as 2013: snail opercula and shells, fiddler crab claws, ribbed mussel, and vegetative matter (sea lettuce).

Pre-existing injuries were observed among 26 (17%) terrapins processed from the trammel net survey in 2014. Carapace (predominantly marginal scute notching) was noted for 19 terrapins, with holes consistent with conical-shaped alligator teeth (Figure 10a) noted for three terrapins; two of these (and six additional) terrapins also had trauma to or were missing at least one limb. Only one alligator sighting (14 October, Figure 10b) occurred during field expeditions in the Ashley River in 2014, but anecdotal reports document alligators in freshwater bodies in close proximity to the Ashley River. As such, and if such data can be available, future research efforts might characterize the diet of culled alligators with an emphasis on terrapin carapace prevalence.





Figure 10. Semi-circular holes (yellow arrows, panel A) in the marginal carapace scutes of diamondback terrapins resembled (shape and spacing) the conical teeth of American alligator (*Alligator mississippiensis*) which were seen twice near Duck Island in 2013 and 2014 (B).

Acoustic telemetry

Acoustic transmitters were attached to 12 diamondback terrapins captured in 2014. Seven of 12 acoustically-tagged terrapins (three male, four female) were captured at three of five Duck Island trammel net stations. The remaining five acoustically-tagged terrapins (two male, three female) were captured at two trammel net stations (AR23 and AR24) across the river from Duck Island.

A total of 21,614 acoustic detections were recorded by 21 VR2W receivers in 2014, of which 76% (16,470) were associated with terrapins acoustically-tagged in 2014. The remainder of receiver detections in 2014 stemmed from seven (of 21) terrapins acoustically-tagged in 2013; last detection for these seven terrapins in 2013 ranged from 8 September to 30 December, with first subsequent detection in 2014 between 8 January and 2 May.

Ninety-five percent (14,187 of 14,963) of acoustic detections for diamondback terrapins captured at Duck Island trammel net stations were recorded by receivers associated with this same area. Similarly, 95% (6,308 of 6,650) of acoustic detections for diamondback terrapins captured at two trammel net stations across and upriver from Duck Island were recorded by a single receiver west of the RT-7 bridge just across and upriver from Duck Island. Given these observations, it is not surprising that monthly mean distance between receivers associated with capture location and detection by other receivers only infrequently exceeded 0.5 km for males or females (Figure 11). These findings collectively suggest high terrapin fidelity to an area, but with tidally-mediated movements within that area. As such, we reject the null hypothesis of that terrapins captured at Duck Island have equal detection probability at Duck Island vs. other areas in the Ashley River.

Seven of 19 acoustically-tagged terrapins made brief excursions away from Duck Island and the opposing shoreline in 2014, 95% of which (433 detections) involved movement down-river. Terrapins were detected at USCG Day Marker #13 between 11 and 29 May (ID 25239) and again between 01 and 02 August (ID 218, 219). Terrapins were detected at the entrance to or within Orange Grove Creek on 01 April (ID 25217) and on 21 days between 15 July and 03 September (ID 217, 219). In contrast, acoustically-tagged terrapin 215 was detected 21 times by a receiver near the I-526 bridge on 11 July. Four single detection events for this and two other terrapins (ID 25237, 25239) were also recorded at this location on 21 and 24 May, 22 June, and 05 September; given prior visits to this location by two of these terrapins and absence from all other receivers during the upriver visit for the third, these single detection events are believable.

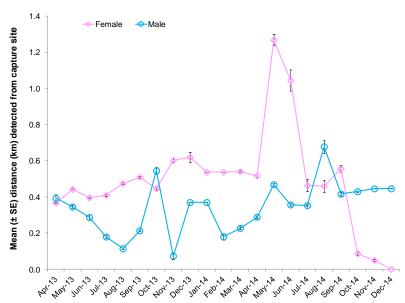


Figure 11. Minimal temporal variability in mean (\pm SE) distance (km) between capture location and other receivers was noted for male (blue line) or female (pink line) terrapins since 2013.

In contrast to nominal temporal variability in distances associated with detection events, seasonal and sex-dependent variability in detection frequency was noted in both study years (Figure 12). For both sexes, monthly detection maxima generally occurred in April and May; the exception for males in November-December 2014 is attributed to over-wintering (or tag loss) within range of two receivers and will be investigated further in winter 2015. Summer and fall detection patterns differed between male and female terrapins in 2013, but were more similar in 2014. Although a high degree of variability was noted in detection frequency among individual terrapins in both years, more similar detection patterns among sexes in 2014 likely reflects increased (38%) receiver coverage in 2014 that facilitated greater detection of females overall.

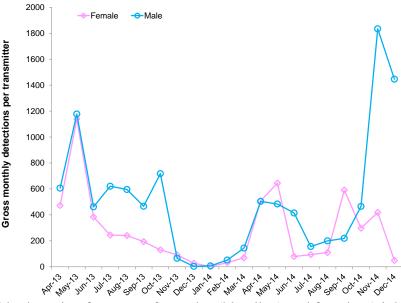


Figure 12. Monthly detection frequency for males (blue line) and females (pink line) was most similar in 2014 which likely reflected 38% more receivers and subsequent female detection.

Monthly mean water temperature (Figure 13a) was significantly correlated (p<0.001, r = 0.99) across years; however, monthly mean salinity (Figure 13b) was not (p = 0.356, r = 0.39). Monthly mean salinity was also not correlated with gross monthly detections for either female (p = 0.590, r = 0.13) or male (p = 0.138, r = 0.34) terrapins.

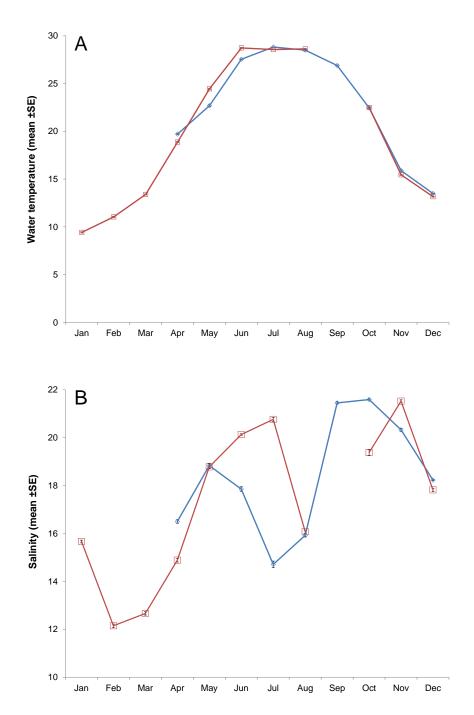


Figure 13. Water temperature (°C, panel A) and conductivity converted to salinity (ppt, panel B) were recorded at 15-minute intervals in the bend of the South Creek of Duck Island. Electrolysis resulted in CTD flooding and loss of all data between 29 August and 13 October.

Job 2. Laboratory observation of BRD effectiveness

Twenty-five female (11.4 to 19.3 cm SCL) and 23 male (10.1 to 13.7 cm SCL) terrapins were used for 192 visual observation trials (~85 hrs) on 12 dates between 8 September and 8 October. Twenty terrapins (10 per sex) were captured by trammel netting in the Ashley River on 10 and 14 April; the remainder of terrapins (15 female, 13 male) were captured by trammel netting in the Ashley River on 26 August. April-captured terrapins were released (near where captured) back in the Ashley River on 24 September, with August-captured terrapins released 10 October.

A total of 11,633 trap contacts were documented during visual observation trials in fall 2014; however, despite this high level of activity, male terrapins did not attempt funnel entry in 28 of 92 trials and female terrapins did not attempt funnel entry in 43 of 100 trials. For both sexes, lack of interest in the funnel entry was greater for funnels outfitted with BRDs than for control traps (Figure 14a), consistent with the terrapin-centric objective of BRDs. Mean tank water temperature during trials was 26.1° C (range = 20.6° C to 29.9° C; n = 361), but was not correlated (p = 0.499, r = -0.22) with trial day lack (% of trials) of funnel interest. Salinity during trials ranged from 12.8 to 16.0 ppt, but was not correlated (p = 0.869, r = 0.05) with funnel interest.

Among terrapins that attempted to enter the funnel, none (13 females, 16 males) were able to successful transit through the reduced height BRD (Figure 14b). Twelve of 13 females that attempted to transit through the vertical orientation BRD were excluded; however, only eight of 14 males (57%) were successfully excluded by this BRD design. In contrast, none of 14 males that approached the standard BRD panel nor any of 20 males that approached control panels were excluded. As predicted based on body size, 11 of 14 (79%) of females approaching the standard BRD were excluded but only two of 17 (12%) approaching the control panel were.

Although these results are preliminary and based on small overall sample sizes, they do suggest a rejection of the null hypothesis that there is no difference in the retention rate of diamondback terrapins in crab traps with and without BRDs, as well as among BRD designs. The reduced height BRD is a superior design for terrapins; however, whether this design deters crab entry remains to be tested under the same controlled conditions. The vertical orientation BRD also appears to be a superior design to the standard BRD given exclusion of all female terrapins and a 57% improvement in the exclusion of male terrapins; however, data on crab impacts are pending.

Job 3. Field study of BRD effectiveness

Thirty-one crab trap fishing days (and 362 trap sets) occurred between 6 May and 5 November. Between 6 May and 17 June, crab trap fishing efforts (54 sets, 15% of total) were primarily focused on the Ashley River and creeks in the vicinity of Duck Island. Crab trap fishing efforts further down river in Orange Grove and Old Towne Creeks was initiated on 30 May and continued extensively through 2 July (plus two additional days in September and October), during which time 116 sets (32% of total) were expended. More than half of all crab trap fishing effort (192 sets) occurred in the Stono River between 17 July and 5 November.

A total of 1,617 hours was recorded for five trap designs as follows: control traps (91 sets, 406.6 hours); standard BRD (90 sets, 404.1 hours); reduced height BRD (90 sets, 401 hours); vertical orientation BRD (86 sets, 399.3 hours); and "toothed" BRD (five sets, six hours). Given great similarity with the standard BRD, testing of the "toothed" design was quickly discontinued.

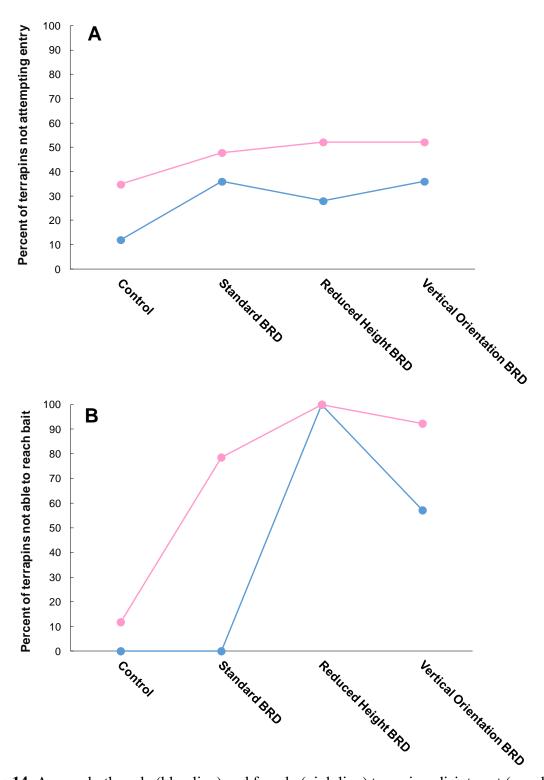


Figure 14. Among both male (blue line) and female (pink line) terrapins, disinterest (panel A) in entry funnels was greatest for controls than for crab trap panels with BRDs. A gradient in terrapin exclusion efficacy (panel B) was observed, with the reduced height opening providing the best overall option, followed by the vertical orientation BRD, and the standard BRD.

A total of 1,341 blue crabs (*Callinectes sapidus*) and four stone crabs (*Menippe mercenaria*) were captured in 290 of 357 non-"toothed" BRD crab sets. Two stone crabs (7.8, 9.7 cm CW) were captured in control traps, the third (8.4 cm CW) was captured in a standard BRD trap, and the fourth (5.1 cm CW) was captured in a reduced height BRD trap.

Blue crabs measured 6.5 to 18.7 cm CW. Complete morphometric data were not collected for 12 blue crabs for various reasons (i.e., broken body parts or crab fell overboard before measuring); however, with certainty four of these crabs were legal-sized (CW ≥12.7 cm, 5"). Morphometric relationships for 31 additional crabs suggested erroneous data for either CW or CL (Figure 15a) and/or CW or BD (Figure 15b). However, because these measurements either reflected accurate CW measurements (i.e., CL or BD inflated) or underestimated CW (i.e., CL or BD correct), none of these crabs were discarded for (a) legal-size catch rate or (b) overall size distribution analyses.

Four hundred ninety-four legal-sized blue crabs (37% of catch) were captured in 205 trap sets. Positive catch rates for legal-sized blue crabs ranged from 0.16 to 1.67 crabs per soak hour. A significant difference ($H_3 = 10.74$, p = 0.013) in legal-sized blue crab catch rates was detected as a result of the reduced height BRD having a median catch value of 0.000 crabs per soak hour vs. 0.227 for the control, 0.204 for the vertical orientation BRD, and 0.184 for the standard BRD.

Frequency of non-capture of legal-size crabs in control traps (37%) was most similar to traps with the vertical orientation BRD (36%, Figure 16). Captures of 0.16 to 1.00 legal-sized blue crabs per hour comprised 53% of control trap sets, most similar to the standard BRD (53%). Due to vertical BRD traps catching 0.16 to 1.00 legal-sized blue crabs per hour more often than control traps, the vertical BRD design was also associated with lower overall occurrence of catch rates >1.00 legal-sized blue crabs per hour (5% of all 357 trap sets) than control traps. It is also worth noting that only the three highest catch rate sets (1.53 to 1.67 legal blue crabs per hour) exceeded the highest catch rate (1.43 legal blue crabs hour) for the vertical orientation BRD.

A significant difference ($H_3 = 18.19$, p <0.001) was also detected in the size distribution of legal-sized crabs between control traps and treatments; the reduced height BRD was associated with the lowest median value (13.9 cm; 73 crabs) vs. 14.6 cm for the control (162 crabs), 14.7 cm for vertical orientation BRD (125 crabs), and 14.9 cm for the standard BRD (130 crabs).

Eleven terrapins (10.9 to 13.1 cm, all male) were also captured in crab traps, with all but one captured in the vicinity of Duck Island on 6 May and 20–21 May; the 11th terrapin, a 10.8 cm male, was captured in the Stono River on 30 July. Five of these terrapins were captured in control traps, three in standard BRD traps, and three in the discontinued "toothed" BRD traps. On 5 June, a 12th terrapin (an 11.6 cm male) was acquired from a crabber in Orange Grove Creek who indicated that other terrapins had also been captured in traps (and died) there recently.

Two of three male terrapins captured in crab traps on 6 May after a one hour soak mysteriously died 24 and 48 hours later. Both terrapins were examined by Dr. Shane Boylan, DVM, at the South Carolina Aquarium; however, a definitive cause of death was not indicated. The third male terrapin captured in the same trap set received an acoustic tag (ID 214) and was detected 3,251 times between 8 May and 15 July, predominantly in the North Creek where it was captured and at AR11 just outside of this creek before detections abruptly ceased.

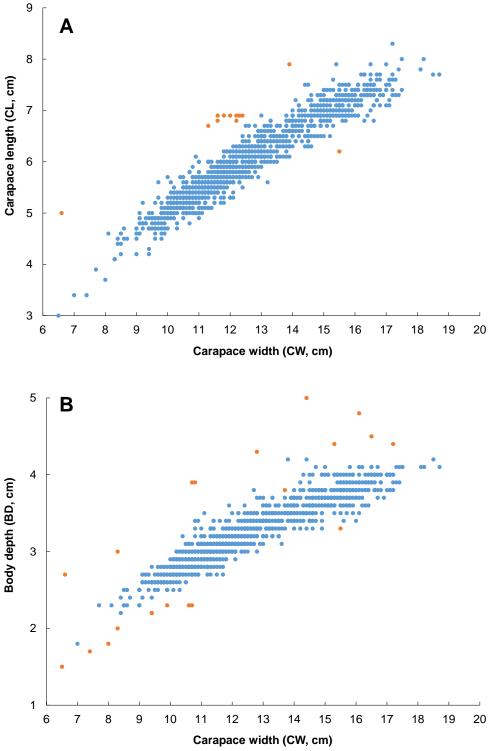


Figure 15. Morphometric relationships between carapace width and carapace length (A) and between carapace width and body depth (B) were documented for 1,329 blue crabs in 2014 in conjunction with evaluating the effectiveness of various terrapin by-catch excluder designs. Orange dots in both panels denote a suspect measurement in each pair that are either resulted in accurate or underestimated measurements for carapace width.

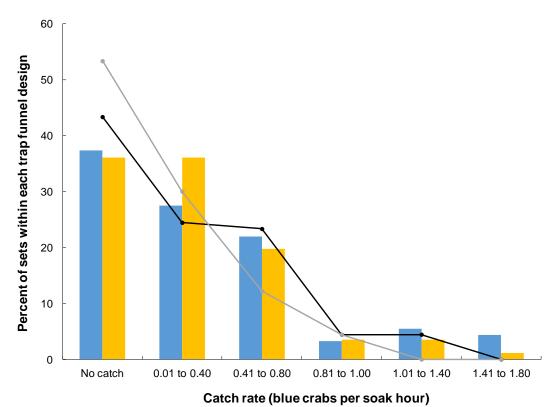


Figure 16. Legal-sized (≥5", 12.7 cm) blue crab catch rates for control traps (blue bar) were significantly different from traps outfitted with reduced height BRDs (gray line), but were not significantly different from standard BRD (black line) or vertical orientation BRDs (orange bar).

Outreach

In January, a synopsis of year one was conveyed to approximately 100 sixth grade students at James Island Middle School. In March, a more advanced version of this information was presented at the SCDNR Marine Resources Division Conference. In June, co-investigators met with Mr. Townsend Clarkson of the Kiawah Island Development Association to discuss potential terrapin research; this meeting in Charleston was succeeded by a meeting on Kiawah that involved numerous entities including biologists from the Town of Kiawah, and we hope to begin testing the vertical orientation design with these groups in 2016. In September, a poster on Job 2 was presented at the Grice Marine Biology Program Graduate Student Colloquium. In October, M. Arendt assisted with terrapin seining at long-term Kiawah monitoring sites now managed by M. Dorcas (Davidson University) and discussed present and future BRD studies. Lastly, several volunteers that assisted with field data collection were marine educators or connected us with educators, providing further opportunity to share highlights from this two-year research study.

In addition to two public re-sightings of tagged animals, we had four public interactions which led to fruitful discussions about terrapin nesting activities near Charleston, SC that will help to shape future research efforts. These interactions ranged from a call about releasing a juvenile terrapin raised from a hatchling after being found near the Battery downtown to reports of contemporary nesting at Charlestowne Landing (Old Towne Creek), the James Island Yacht Club (Charleston Harbor), and the St. John's Marina in the Stono River.

During crab trap fishing and other terrapin field days, we also discussed the research with six different crabbers in the Ashley River and four different crabbers in the Stono River; one of these interactions led the donation of a crab trap-captured terrapin.

Collaboration

Mating in the holding tank inside the Sea Turtle Hospital at the SCA occurred shortly after transport of 10 male and 10 female terrapins collected from the Ashley River in April. Nine of 10 female terrapins ultimately laid 81 eggs, which were incorporated into a companion study also funded by the U.S. Fish and Wildlife Service (State Wildlife Grant) to evaluate growth rates of young of the year hatchlings reared under various diet regimens (Levesque and Gross – PIs).

In addition to terrapin detections, acoustic receivers also recorded 89 detections for a southern flounder tagged in a N. Carolina study managed by F. Scharf (UNC Wilmington) and 35 detections for an Atlantic sturgeon tagged in a S. Carolina study managed by B. Post (SCDNR); detection data were promptly provided to these researchers following receiver uploading.

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At the South Carolina Aquarium, we thank Christi Hughes and Whitney Daniel for husbandry assistance in support of Job 2 of this research study, as well as Dr. Shane Boylan, DVM, for continued medical support and diagnostic capabilities as needed. We also thanks Janelle Johnson (CofC) for taking the lead on initiating and sticking with Job 2 data collection.

Invaluable logistical support was provided by Ellen Waldrop and Brooke Czwartacki, initially as volunteers in winter 2014 and then as a SCDNR employees beginning in spring. We also thank the following SCDNR employees for assisting with field data collection: Jessica Johnson, Sean Miller, Lauryn Wright, Kayla Spry, Catharine Parker, George Reikerk, and Adam Lytton. We also thank the following SCDNR employees for guiding us into the world of crab trap fishing: Stephen Long, Larry Delancy, and Amy Fowler.

More than 270 volunteer hours were logged by 13 individuals in support of this research in 2014, which allowed our nominal staff to complete multiple tasks across this and other granting needs. We thank the following volunteers for their valuable contributions in 2014: Kateyln Andrea, Melissa Johnson, Shelley Dearhart (SCA), Emily Shaw and Courtney Corvino (Charleston Southern University), Jenna Quinn (CofC), Joanna Reinhold (Wake Forest University), Theresa Cantu (MUSC), Dakotah Merck (USC), Kevin Kurtz (educator), and Kristen Gold.

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Appendix 1. Ashley River Trammel Net Station Zones (courtesy of A. Grosse).

